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Research Article

A STUDY ON THE RELATIONSHIP BETWEEN THE RATE OF CONCEPTION IN ARTIFICIAL INSEMINATION AND THE MECHANICAL ENERGY OF SPERM

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ABSTRACT

BACKGROUND: The mechanical energy of sperm is an optimal index with which to quantitatively assess the motility of sperm. The sperm energy index (SEI) is proposed as an index representing the total mechanical energy of sperm in a single field when measured using computer-assisted sperm analysis. The mechanical energy of a single sperm is given by (SEI/motile sperm count) × 100 which has been designated S-SEI.

PURPOSE: The aim of the current study was to examine the relationship between SEI or S-SEI and the rate of conception in intrauterine insemination (IUI).

METHOD: The materials used were 1,006 semen samples from 1,006 cycles of artificial insemination with the husband's semen (AIH). Artificial insemination was undergone by 367 couples. The SEI and S-SEI were determined using a prepared suspension of sperm prior to IUI.

RESULTS: The average rate of conception for all samples was 7.5%. The rate of conception increased as the SEI increased, and the rate of conception was consistently 9.95% at an SEI of 9.5 or higher. The rate of conception peaked at 9.7% in the range of 1.6<=S-SEI<2.1. Samples with an SEI>=9.5 and 1.6<=S-SEI<2.1resulted in the highest rate of conception10%.

CONCLUSIONS: A combination of the SEI and S-SEI, which are indices of the mechanical energy of sperm, could serve as a criterion to determine whether or not a couple will be able to conceive through AIH.

INTRODUCTION

Several studies have reported intense activation of sperm called hyperactivation prior to fertilization (Morales *et al*.,1908; Yanagimachi,1970), and several have described the increased motility of sperm as a result of the addition of pentoxifylline (PTX)(Dimitriadou *et al*.,1995; Guasti *et al*.,2013). Studies using computer-assisted sperm analysis (CASA) have revealed that in increase in the curvilinear velocity (VCL) of sperm or the amplitude of lateral head displacement (ALH) and a decrease in the linearity (LIN) of the path of sperm cause sperm to be more motile (Cancel *et al*.,2000; Schmidt and Kamp,2004; Mortimer *et al*.,1990). However, these parameters and the percentage and concentration of motile sperm cannot equivalently quantify the level of sperm motility.

Over the past few years, the laws of physics governing the VCL and the ALH of sperm and the laws of physics governing the curvature of the path followed by sperm have been determined.

The equation for sperm motility, derived from the sperm spring theory (Isobe, 2007), and inequality of the curvature of the path followed by sperm, derived from the sperm curvature theory (Isobe and Matsuura,2008), are shown below. A is the ALH, V is the VCL, and $\boldsymbol{L}$ is the LIN. a, b, and c are constants.

Equation for sperm motility (Sperm equation)

$$\left(\frac{1}{V}\right)=a\left(\frac{1}{A}\right)+b$$

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Inequality of the curvature of the path followed by sperm $L^2<1-cV^2$

In line with the laws of physics, the motion of an object can be commensurately quantified as its mechanical energy (the sum of its kinetic energy and potential energy). Thus, the mechanical energy of a single sperm can be used to quantitatively assess the motility of a single sperm, and the total mechanical energy of all sperm in a group is the optimal way to quantitatively assess the motility of that group.

The sperm energy index (SEI) is proposed as an index representing the total mechanical energy of sperm in a single field when measured using CASA. SEI has been derived from new sperm energy theory (Isobe,2012). SEI represents the total mechanical energy of the sperm existing in a single visual field of CASA. The SEI is defined as follows.

$$SEI \equiv \frac{nK\lambda}{100}$$

Here,λ is the mean value of the square of the ALH for all sperm in a single visual field of CASA, and n is the number of motile sperm in a given field. K (Isobe Coefficient) is a specific constant which fulfills the functional equation of sperm existence probability density with respect to the ALH below. Isobe coefficient can be obtained by minimizing the standard error between the actual and theoretical distribution of the probability density of sperm (Isobe,2016).

$$P = \frac{C}{A\left(n\lambda - A^2\right)^{\frac{1}{2}+nK\lambda}} \left\{ \frac{n\lambda - A^2}{(n-1)A^2} \right\}^{KA^2}$$

Here, A is ALH, C is a constant by which P value integrated by A in all range becomes 1.

The mean mechanical energy of a single sperm can be obtained by dividing SEI by the motile sperm count n. The single sperm energy index (S-SEI) is defined as follows:-

$$S-SEI \equiv \frac{SEI}{n} \times 100 = K\lambda$$

This value is multiplied by 100 to shift the decimal place.

Recently, a study used the S-SEI to quantitatively assess changes in the motility of sperm over time (Ohnishi *et al.*,2018). In that study, a suspension of sperm treated with Percoll and a suspension of sperm treated using the swim-up technique were left at normal temperature, the suspensions were heated to 37°C, and PTX was added to each suspension. Results confirmed that the S-SEI indicates the mechanical energy of a single sperm in fact.

The aim of the current study was use the SEI and S-SEI to quantitatively assess the relationship between the rate of conception in intrauterine insemination (IUI) and the mechanical energy of sperm.

MATERIALS AND METHODS

The materials used were 1,006 semen samples from 1,006 cycles of artificial insemination with the husband's semen (AIH). Artificial insemination was undergone by 367 patients (in couples), and the procedure was performed at this facility from September 2016 to December 2017. The relationship between the rate of conception in IUI and the SEI and S-SEI was examined. The SEI and S-SEI were determined using a prepared suspension of sperm.

Ovarian follicular development was the result of a natural cycle or it was promoted with clomiphene at a dose of 50–100mg for 5 d or intramuscular injection of FSH or hMG. The diameter of ovarian follicles was tracked with transvaginal ultrasound. When the diameter of the dominant reached 18mm, 5,000 units of hCG was administered to induce ovulation. At around ovulation, a suspension of sperm with its volume adjusted to 0.3ml using 90% Percoll was injected into the uterus. There was no prescribed rest period after injection. If ultrasound revealed the presence of a fetal sac, the patient was deemed to be pregnant. Parameters of sperm motility were measured immediately prior to insemination into uterus at room temperature using a newly generated sperm motility analysis system (SMAS Ver.3.00) which is a Japanese CASA system (DITECT, Tokyo, Japan). Five μℓ aliquots were placed in a Makler chamber (Makler,1980) immediately prior to insemination. A Makler chamber is suitable for calculation of the SEI. The equation for sperm motility is the basis for deriving the SEI. In a previous experiment using chambers with various depths, this sperm equation was most accurately obtained with a Makler chamber(Isobe,2009).

The SEI was calculated by minimizing the standard error between the actual and theoretical distribution of the probability density of sperm with respect to the ALH. The SMAS allows measurements to be processed with Excel (Microsoft), so the SEI can be calculated using that Excel program (DITECT, Tokyo, Japan).

SMAS version3 has a resolution of 52 × 10^5 pixels (1pixel = 0.4752761 μm) and a frame rate of 60 Hz (a total of 60 frames captured per second). SMAS setting were done as follows: Images were captured with a 10×objective, sperm were detected at a size of 8–80 pixels, cell intensity was 50, and sperm were identified as immotile within a range of 4.45 pixels. The cut-off for swimming sperm was an average path velocity (VAP) of 25 μm/s and a straightness (STR= VSL/VAP) of 70%. A hard drive containing recorded video of specimens from DITECT (Tokyo) was used for internal quality control of sperm motility.

A ratio test with a significance level of 5% was used to compare pregnancy rates. An unpaired t-test with a significance level of 5% was used to compare patient's ages.

RESULTS

The average rate of conception for all samples was 7.5% (75/1,006). Figure1-1 shows all of the samples in order of ascending SEI. The rate of conception was examined by stratifying the SEI into 4 strata, 5 strata, or 7strata. Figure1-2 shows all of the samples in order of ascending S-SEI. The rate of conception was examined by stratifying the S-SEI into 4 strata, 5 strata, or 7strata (patients in each stratum were classified as group 1, group 2, group 3… based on whether they had a smaller SEI or S-SEI).

The rate of conception remained constant for groups 3-5 in the 5 strata of the SEI. In other words, the rate of conception increased as the SEI increased, and the rate of conception was consistently 9.95% (20/201) at an SEI of 9.5 or higher. A likelihood ratio test with a significance level of 5% revealed significant differences in the SEI for group 1 and group 3(P=0.005). In the 4 strata and 7 strata of the S-SEI, group 2 had the highest rate of conception. In other words, the rate of conception peaked at 9.7%(14/144) in the range of 1.6<=S-SEI<2.1. In the 7 strata of the S-SEI, the S-SEI differed significantly in group 1 and group 2(P=0.016). Samples with an SEI>=9.5 and 1.6<=S-SEI<2.1resulted in the highest rate of conception (10%, 8/80).

The WHO laid out criteria for evaluation of semen in 2010. Based on those criteria, ejaculate samples with a semen volume<1.5ml, a concentration of sperm<15×10^6/ml, a percentage of motile sperm<40%, or a total number of sperm<39×10^6 were deemed to be samples with a male-related factor for infertility. The proportion of sperm samples with a male-related factor out of all samples is shown in Fig. 2. Figure 2-1 shows 5 strata in order of ascending SEI(just like the 5 stratum in Fig. 1-1) for the male-related factors, and Fig. 2-2 shows 7 strata in order of ascending S-SEI(just like the 7 strata in Fig. 1-2). Samples were more likely to have a low percentage of motile sperm if they had a lower SEI. Only samples from group 1 were likely to have a low percentage of motile sperm in the strata in order of ascending S-SEI. Samples with a low concentration of sperm and samples with few sperm were more likely to have a lower SEI, but a low concentration of sperm and few sperm were not correlated with the S-SEI. A low volume of semen was not correlated with the SEI or S-SEI.

The average age and standard deviation (SD) for couples in the 5 strata in order of ascending SEI are shown in Fig. 3-1. The average age and SD for couples in the 7 strata in order of ascending S-SEI are shown in Fig. 3-2. Significant differences among strata in terms of the order of ascending SEI and ascending S-SEI were not noted (Data not shown).

DISCUSSION

Recently, a study reported that addition of PTX at a final concentration of 1mg/ml resulted in an increase of the S-SEI by 1.2(Ohnishi *et al*.,2018). Based on that finding, the treatment strategy for a couple with an S-SEI lower than 0.4 should probably be changed to in-vitro fertilization. Results of the current study can serve as criteria with which to predict the rate of conception in IUI. The rate of conception in IUI peaked when the S-SEI was in a certain range (this phenomenon has been designated the “bell-net” effect). In the current study, 85.3%(64/75) of the patients who conceived did so through pre-ovulation IUI while 14.7%(11/72) did so through post-ovulation IUI. In addition, 76.0%(57/75) of patients were underwent insemination into uterus and administration of 5,000 units of hCG prior to ovulation in the same time. Thus, a discrepancy in the timing of an hCG trigger injection or injection of prepared sperm into the uterus may have resulted certain values in the identified range. The reasons for the “bell-net” effect are unclear and are a topic for future study.

PTX is a cyclic AMP (cAMP) phosphodiesterase inhibitor. Adding PTX to a suspension of sperm presumably sustains the level of cAMP in sperm, thus sustaining the motility of sperm(Calogero *et al*.,1998). Adding PTX to thawed sperm once they are frozen and to tissue samples from testicular sperm extraction (TESE) has been used to activate immotile sperm for use in intracytoplasmic sperm injection (ICSI) (Kovacic *et al*.,2006). A study added PTX at concentration of 1mg/ml to a suspension of sperm treated with Percoll at 37^0C has been done using the S-SEI to quantitatively assess changes in the activation of sperm over time (Ohnishi *et al*.,2018). Immediately after PTX was added, the S-SEI increased by 1.2 and the mechanical energy of a single sperm increased by 1.3-fold in comparison to the S-SEI for a suspension of sperm at normal temperature. The increased S-SEI and the increased mechanical energy of a single sperm persisted for at least 90 min. One study reported that excessive addition of PTX decreased the survival rate of sperm (Matsuura *et al*.,2008), and another reported that the level of motility was highest when the concentration of PTX was 2.8mM/ℓafter addition(=0.78mg/ml;molecular weight of PTX: 278.3)(Paul *et al*.,1996). Instead of increasing the mechanical energy of a single sperm, the concentration of motile sperm can be increased to increase the SEI. The Percoll treatment in a 2-layer Percoll density gradient can be changed to increase the concentration of motile sperm (Matas *et al*.,2011). A study has also reported that acupuncture increases the SEI in ejaculate (Isobe,2014). In the current study, prepared semen from patients with asthenospermia was likely to be found in the first stratum of the strata in order of ascending S-SEI as shown in Fig. 2-2. Adding PTX to a final concentration of 1mg/ml resulted in the S-SEI increasing by 1.2, thus pushing the sample into the second stratum. This is likely to have led to an increased rate of conception. Determining the SEI and S-SEI for a prepared suspension of sperm allows a decision as to whether the suspension needs to be concentrated again or whether PTX needs to be added. Studies have reported that adding PTX increases the rate of conception in IUI (Tarlatzis *et al*.,1995; Mehrannia,2009). Nonetheless, determination and adjustment of the SEI and S-SEI might further increase the rate of conception.

Disclosures

This study was conducted in compliance with the Helsinki Declaration. This research project has been approved by a suitably constituted Ethics Committee. This study was approved by the Institutional review board of this facility. All patients provided consent to all treatment procedures and agreed to anonymous use of their data for studies, and obtained data were kept secure at this facility in a form in which patients could not be identified individually.

The current authors had no conflicts of interest with regard to this study. This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Figure legends

Figure 1 Relationship between the mechanical energy of sperm and the rate of conception in IUI

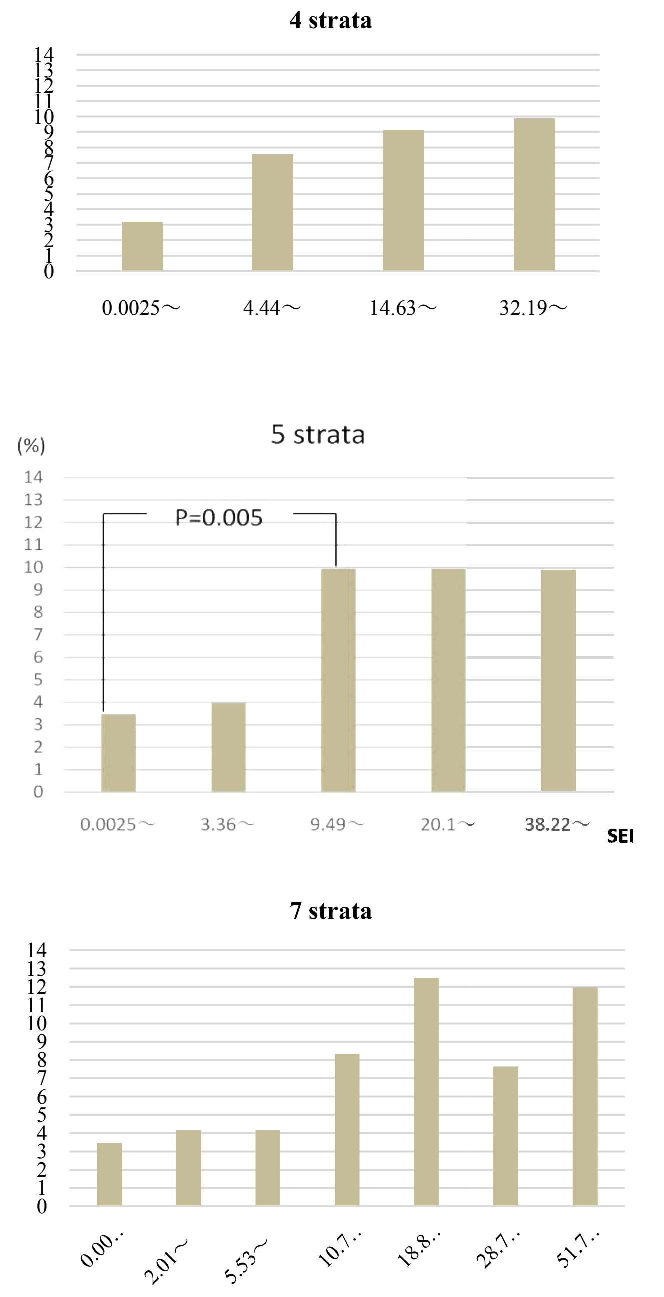

Fig.1.1

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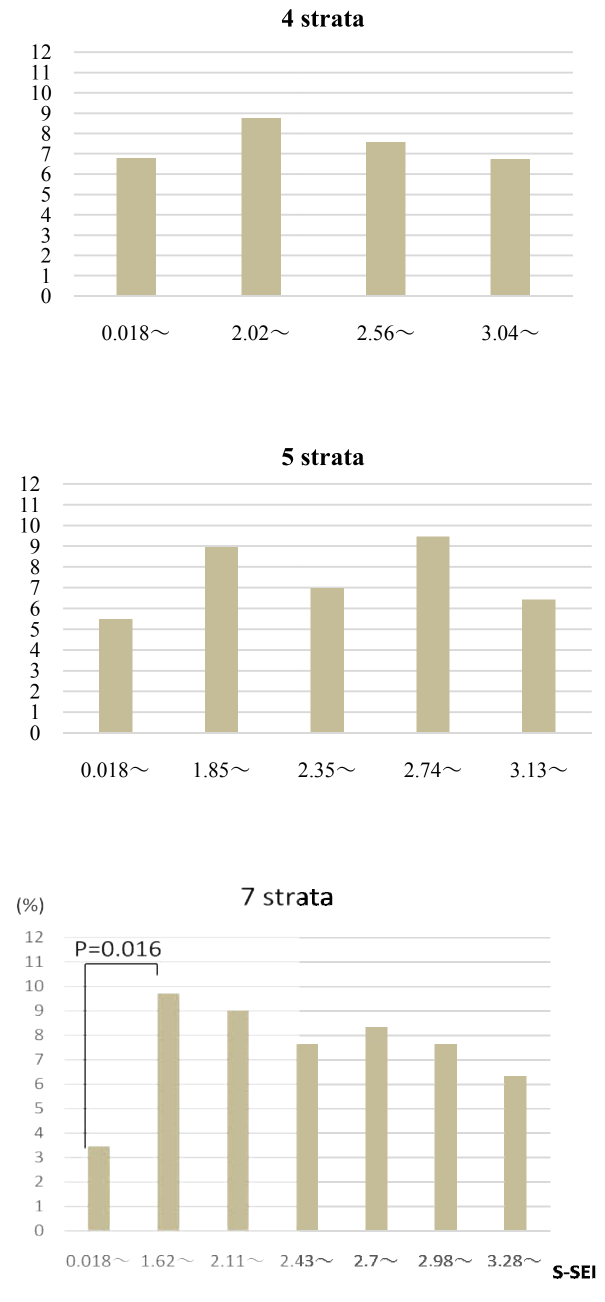
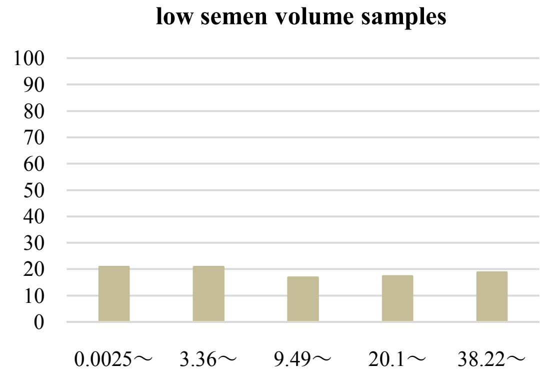
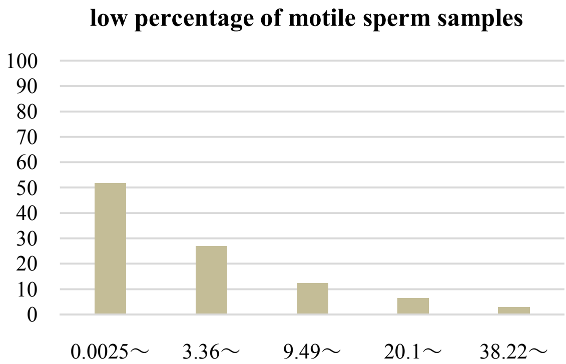


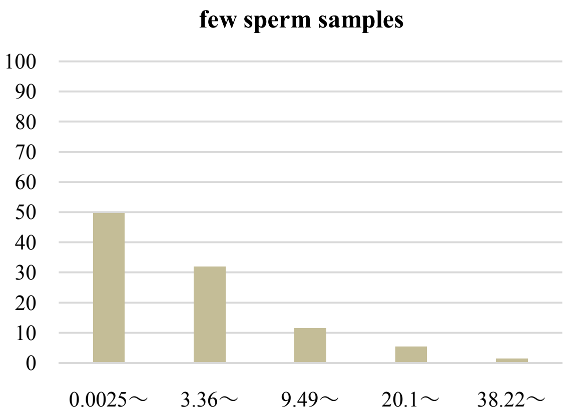
Fig.1.2

Figure 2 Proportion of samples with a male-related factor by strata in order of ascending SEI and S-SEI

The WHO laid out criteria for evaluation of semen in 2010. Based on those criteria, ejaculate samples with a semen volume<1.5ml, a concentration of sperm<15×10^6/ml, a percentage of motile sperm<40%, or a total number of sperm<39×10^6 were deemed to be samples with a male-related factor for infertility. The proportion of sperm samples with a male-related factor out of all samples is shown. Figure 2-1 shows 5 strata in order of ascending SEI(just like the 5 stratum in Fig. 1-1) for the male-related factors, and Fig. 2-2 shows 7 strata in order of ascending S-SEI(just like the 7 strata in Fig. 1-2). Samples were more likely to have a low percentage of motile sperm if they had a lower SEI. Only samples from group 1 were likely to have a low percentage of motile sperm in the strata in order of ascending S-SEI. Samples with a low concentration of sperm and samples with few sperm were more likely to have a lower SEI, but a low concentration of sperm and few sperm were not correlated with the S-SEI. A low volume of semen was not correlated with the SEI or S-SEI.







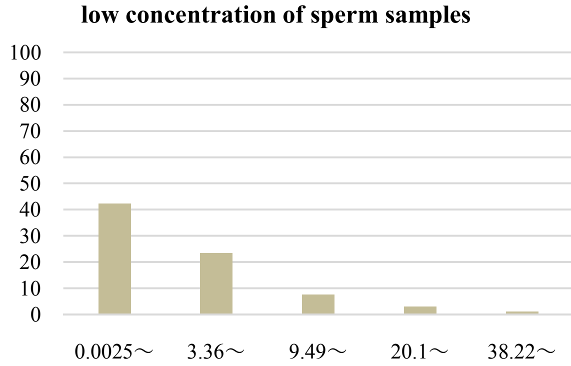
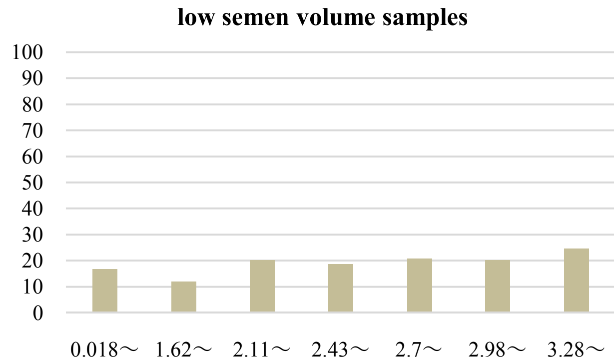
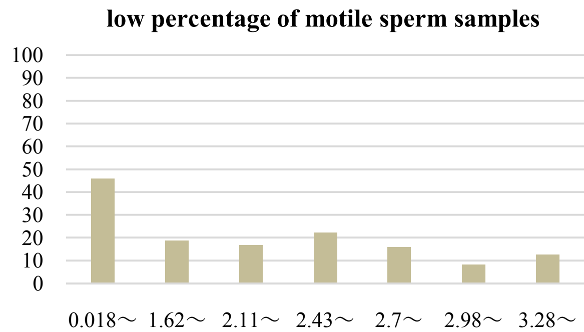
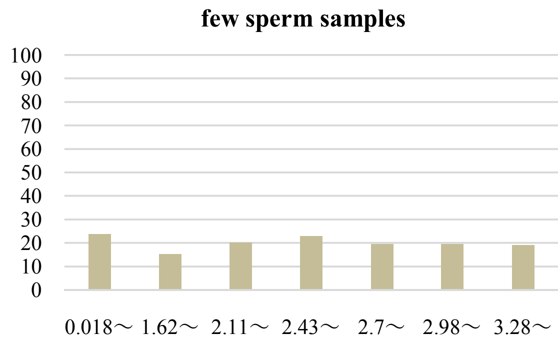


Fig.2.1







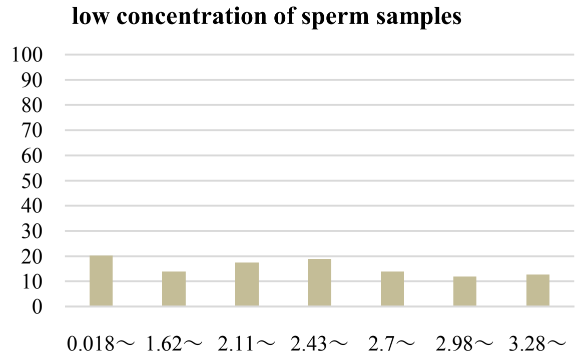
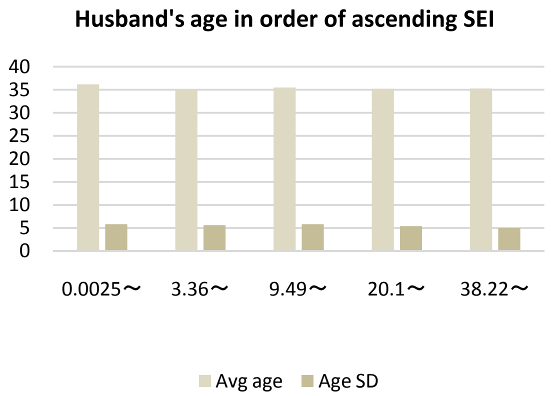


Fig.2.2



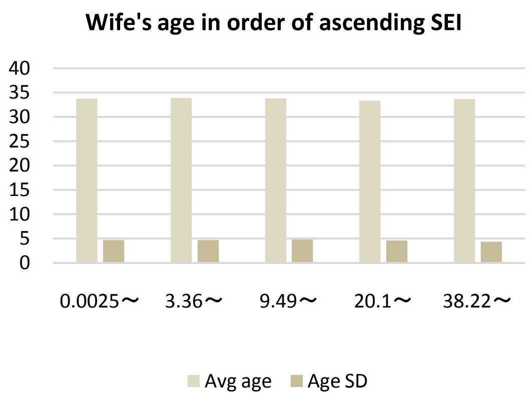
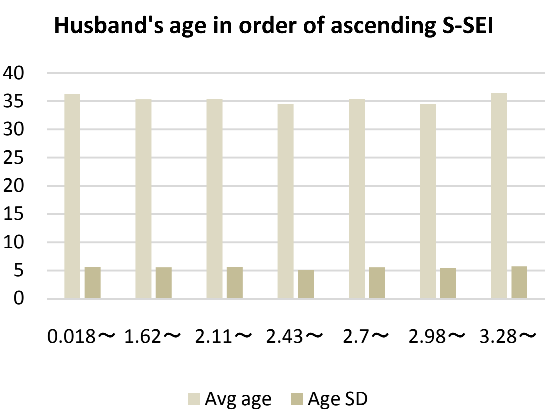


Fig.3.1



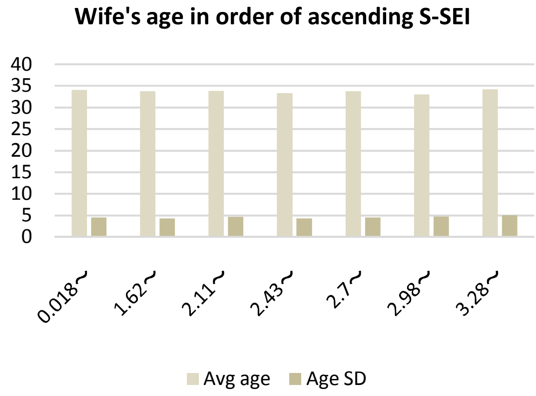


Fig.3.2

Figure 3 Age of couples by strata in order of ascending SEI and ascending S-SEI

The average age and standard deviation (SD) for couples in the 5 strata in order of ascending SEI are shown in Fig. 3-1. The average age and SD for couples in the 7 strata in order of ascending S-SEI are shown in Fig. 3-2. Significant differences among strata in terms of the order of ascending SEI and ascending S-SEI were not noted.

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